CHARACTERISTICS OF GROWTH AND GAS EXCHANGE OF THE ALGA ANACYSTIS NIDULANS IN INTENSIVE CULTURE

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When the choice of photoautotrophic organisms is being made for life support systems, the main requirement is high intensity of their photosynthetic processes and resistance to various external environmental factors. /480

The blue-green algae are of considerable interest from this point of view. As the most primitive photoautotrophic organisms, the blue-green algae exhibit unusual physiological and biochemical variety connected with the great plasticity of their organization. This enables the blue-green algae to live under extreme conditions [2, 7] and thus provides a wide range of forms possessing the most useful physiological and ecological characteristics.

Contrary to widespread views, the literature now contains many facts indicating the high physiological activity of certain known forms of blue-green algae [8, 10].

One such form is Anacystis nidulans. When under conditions of intensive movement this unicellular alga is shaped like a short cylinder or rod measuring $1.3 \pm 0.2 \times 2.5 \pm 0.2\ \mu$. This form of alga was generously presented to us by V. V. Pinevich (Biological Institute, Leningrad University). /481

The object of the present investigation was to continue the search for new forms of algae for use in photosynthetic gas exchanges. We investigated the productivity of this culture when grown under intensive conditions, the characteristics of its gas exchange, and the chemical composition of accumulated organic matter.

The culture of A. nidulans was grown on Arnon's medium. This medium was recommended to us by the Leningrad Biological Institute. After comparative studies of the rate of growth of a culture on the media of Arnon, Kratz and Myers [10], and Takha [6], used for cultivation of blue-green algae, Arnon's medium was found to be most suitable for intensive cultivation of the algae because of its high concentration of mineral elements.

The composition of this medium was as follows (in g/liter): KNO_3 2.02; K_2HPO_4 0.35; $MgSO_4$ 0.25; KCl 0.3; NaCl 0.12; $FeSO_4$ 0.025; $CaSO_4$ 0.09; EDTA 0.05. Trace elements were added at the rate of 1 ml/liter medium.

Before the experiment the culture was grown in a luminostat until its density was 0.2-0.5 g/liter, with an intensity of illumination of 7-8 klux and at a

temperature of 37-40°, ventilated with a mixture of air and carbon dioxide. The culture was then centrifuged at 5000 rpm and resuspended in fresh nutrient medium with a single or double concentration of elements, after which the suspension was transferred into the experimental reactor.

The reactor consisted of a rotating cylinder with its outer surface illuminated by 3N-8 incandescent lamps. The intensity of illumination in the experiments varied from 20 to 60 klux depending on the density of the suspension. The experimental system included a closed air circuit with a volume of 75 liters, in which the circulation of air was produced by a membrane pump. The CO_2 concentration in the air circuit of the system was maintained between 1 and 5%. In these experiments the main conditions of cultivation which had previously found to be optimal for Chlorella when grown in the same reactor were maintained. The air circuit of the system also included an automatic recording gas analyzer (Uras) for measuring the CO_2 concentration in the gaseous phase of the system. The CO_2 and oxygen concentration were periodically checked with Haldane's apparatus. The quantity of CO_2 dissolved and fixed in the suspension was determined with Van Slyke's apparatus and the pH of the suspension was measured potentiometrically.

The intensity of photosynthesis of the algal culture was assessed by the rate of CO_2 consumption, the rate of O_2 liberation, and the rate of accumulation of biomass. The increase in biomass was determined from the dry weight of the centrifuged and washed mass of cells. The elementary composition of the dry material was determined by chemical analysis. Ash was also determined quantitatively and qualitatively. In the course of the experiment the nitrogen concentration in the medium was checked. /482

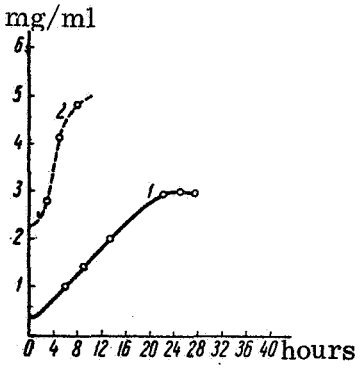

Figure 1. Dynamics of Growth of A. nidulans in Intensive Culture with Initial Density 0.35 (1) and 2.2 (2) mg/ml.

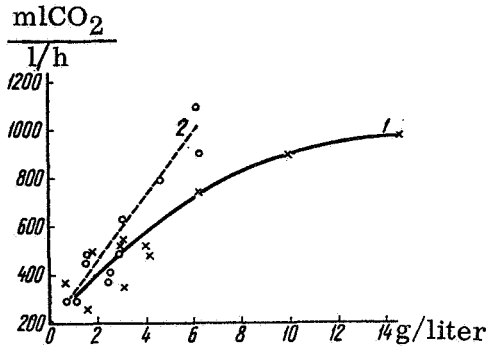

Figure 2. Comparative Characteristics of Rate of CO_2 Absorption in Suspensions of Chlorella (1) and Anacystis (2).

The characteristics of the rate of growth of the culture are illustrated in Fig. 1. Curve 2 shows that with a fairly high initial density the rate of increase

of biomass over the linear portion of the curve is 0.65 g dry substance/liter/h, close to the productivity of the most active thermophilic forms of Chlorella cultivated under the same conditions.

The rates of absorption of CO_2 by Anacystis cultures of different densities are shown in Fig. 2 (Curve 2). For comparison, the corresponding values for Chlorella pyrenoidosa S-39 obtained with analogous conditions of cultivation in the same reactor [4] are given (Curve 1).

It is clear from Fig. 2 that with suspensions of density of the order of 1-3 g/liter, the productivity per unit volume of suspension of the compared forms of algae is practically identical.

With a greater density of the suspension (6-7 g/liter) and a corresponding increase in illumination, the productivity of the Anacystis suspension per liter was found to be higher than that of Chlorella.

This should not be taken to mean that the absolute productivity of Anacystis is greater, because for equal densities by weight, the Chlorella suspension contains fewer cells. However, the smaller size of the Anacystis cells may be /483
another advantage, because intensity of metabolism is proportional to total surface area of the cells.

Hence, like Chlorella, Anacystis can maintain a forced cultivation regime. Its productivity during preliminary trials under these conditions reached 26 liters CO_2 per liter suspension per day.

The relationship between the quantity of CO_2 absorbed by the cells and the quantity of oxygen liberated was also studied in these experiments. The results are given in Table 1.

TABLE 1. Relationship between CO_2 Absorbed and O_2 Liberated during Intensive Cultivation of A. nidulans

Volume of CO_2 absorbed liters	Volume of oxygen liberated, liters	CO_2/O_2	Volume of CO_2 absorbed liters	Volume of oxygen liberated, liters	CO_2/O_2
0,785	1,125	0,70	0,820	1,120	0,73
0,825	0,975	0,84	0,150	0,220	0,67
0,900	1,200	0,75	0,510	0,675	0,72
2,850	3,970	0,72	0,525	0,825	0,90
1,010	1,350	0,75	1,050	1,350	0,78
1,200	1,420	0,84	1,200	1,420	0,84
2,620	2,850	0,92	2,250	2,550	0,88
0,450	0,600	0,75	2,550	2,850	0,89
			Mean		0,79

Commas represent decimal points.

The results given in Table 1 show that the CO_2/O_2 ratio (the accumulation coefficient) varies from 0.70 to 0.90, with a mean value of 0.79. The effect of the nitrogen food supply on fluctuations in the assimilation coefficient of Anacystis is worth noting. After complete exhaustion of nitrate nitrogen from the medium (the appearance of NO_2^- or NH_4^+ ions in the medium was not observed during cultivation), growth of the culture continued without any appreciable decrease in rate (Fig. 3). The process of depletion of nitrogen in the medium, when calculated per gram of biomass formed, shows considerable irregularity in the case of Anacystis in contrast to Chlorella and Scenedesmus. As an example, data for nitrogen depletion in the medium are given in Table 2.

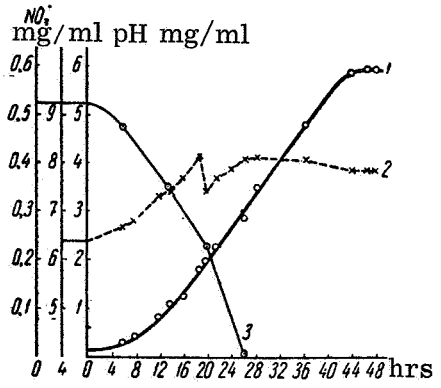

Figure 3. Dynamics of Increase of Biomass (1), pH of Medium (2) and Depletion of Nitrogen in Medium and (3) in a Culture of Anacystis.

Usually a steep drop is observed in nitrogen depletion in most cases during the first hours of the experiment, and sometimes in the middle of the experiment. As a rule this drop is followed by a decrease in the consumption of nitrogen from the medium by the cells.

The mean loss of nitrogen from the medium, calculated per gram of dry substance at the moment of its total disappearance from the medium was fairly stable, amounting to 120-150 mg/g depending on its initial concentration in the medium. If the loss of nitrogen from the medium is related to the total increase of biomass before the curve flattens out on the plateau, a value for the relative loss of nitrogen of 70-90 mg/g dry substance is obtained, which is very close to the extraction of nitrogen by Chlorella. The quantity of nitrogen found in the dry substance of the cells is the same as its mean extraction from the medium when the cultivation curve becomes a plateau. The utilization of extremely large quantities of nitrogen in the initial periods of growth and maintenance of a high rate of growth of the culture when mineral nitrogen is absent from the medium reflect distinctive features of the cell metabolism of blue-green algae. There are reports in the literature that the cells of these algae accumulate large quantities of soluble organic forms of nitrogen, foremost among which are diamino acids and amides [1, 11]. /484

The dynamics of the pH of the suspension during cultivation of Anacystis is also shown in Fig. 3. With an increase in the biomass the pH moved toward the alkaline side from 5.3 to 7.9 in single-strength medium and from 4.5 to 8.1 in double-strength. The change in pH over this range was not reflected in the rate of growth of the culture. The increase in pH of the suspension was accompanied by accumulation of fixed CO_2 to 0.6 - 0.7 cm^3/ml. Near the time when all NO_3^- ions had disappeared from the medium the pH and concentration of bicarbonates in the suspension were stabilized at the same level. /485

TABLE 2. Rate of Increase of Biomass and Extraction of Nitrogen from Medium during Intensive Cultivation of A. nidulans

Time, h	Density of suspension, g/liter	Nitrogen concentration, mg/liter	Extraction of nitrogen, mg/g biomass	Time, h	Density of suspension, g/liter	Nitrogen concentration, mg/liter	Extraction of nitrogen, mg/g biomass
Single strength Arnon's medium				Double strength Arnon's medium			
0	0,25	300	—	0	0,15	525	—
4	0,40	272	182	8	0,45	460	216
8	0,84	230	95	12	0,81	390	195
12	1,44	166	151	16	1,35	305	120
16	2,04	66	166	20	2,05	220	121
20	2,64	8	97	24	2,75	110	143
21	2,76	0	66	28	3,50	0	133
During 21 h	2,80	0	117	During 28 h	3,50	0	156

Commas represent decimal points.

TABLE 3. Elementary Chemical Composition of Biomass of A. nidulans (% of dry weight)

Experiment No.	N	P	Na	K	Ca	Mg	Fe
1	6,82	0,77	0,05	0,03	0,15	0,24	0,14
2	8,00	1,20	0,04	0,07	0,26	0,40	0,09
3	8,62	0,96	0,04	0,05	0,25	0,25	0,08
4	8,19	1,40	0,04	0,03	0,13	0,30	0,08
5	8,00	1,10	0,04	0,06	0,21	0,24	0,06
6	7,91	1,43	0,03	0,04	0,18	0,30	0,09
Mean	7,92	1,13	0,04	0,05	0,20	0,29	0,09

Commas represent decimal points.

It was also interesting to determine the elementary composition of the organic matter of the cells, to some extent characteristic of their biochemical composition.

For this purpose a washed and dried biomass obtained from experiments when the growth curve had come out onto a plateau was used. The ash content constituting on the average 4.6% of the dry weight, and its composition were determined in the biomass (Table 3).

The carbon content in the organic matter, expressed as a proportion of the ash-free residue, was 51.44%, the hydrogen content 7.39%, nitrogen 8.30% and oxygen 32.87% (mean results of fixed determinations).

Using these data as a starting point, the empirical formula of the organic matter of which the cells of A. nidulans are formed was calculated, the result being $C_{7.22}H_{12.40}O_{3.46}N$.

The reducing power of the organic matter (R), calculated by means of Milner's equation [12], is 40.81. This value is rather higher than for Chlorella [5].

These reulsts show that in its elementary chemical composition the biomass of Anacystis is similar to that of Chlorella, from which it differs mainly in its lower content of monovalent cations K and Na [3]. The content of the principal biogenic element, carbon, hydrogen, oxygen and nitrogen, expressed relative to the content of organic matter, likewise showed no significant difference from their content in Chlorella. /486

The results of these investigations showed that Anacystis nidulans can be used in intensive cultivation as well as Chlorella. A further study of the nitrogen feeding requirement and the associated metabolic processes in the cell may reveal the possibility of controlling the assimilation coefficient and hence the composition of the biomass of this alga.

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